

Factors influencing the proximate composition of milk in a sub-polar otariid, *Callorhinus ursinus*

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Abstract

Milk lipid is the primary energy source for provisioning pinniped offspring. The percent lipid in milk varies considerably for the taxon and within individual species with important implications to reproductive energetics, life-history strategies, maternal investment, and population energy models. For otariid milk, days postpartum and time on shore (i.e. length of the suckling bout) are known to affect percent lipid. Cross species comparisons indicate longer trip durations may increase percent lipid as well; intra-species correlations, however, remain equivocal. Diet has also been implicated in influencing total energy transferred to offspring. We used multiple linear regression models to test the significance of days-postpartum, time on shore, preceding trip duration, maternal mass, dive type (as a proxy for diet), island and year in determining the proximate composition (lipid, protein, water, and ash) of northern fur seal milk. Milk samples (n=189) were collected from 101 radio-transmittered northern fur seals in the Pribilof Islands from July through October in 1995 and 1996. Total percent lipid (Roese-Gottlieb) ranged from 28.5-66.8% (mean: 49.0±0.56) and the calculated gross energy ranged from 13.05- 27.98 kJ·g⁻¹ (mean: 21.26±0.21). Trip durations ranged from 2.5-14.1 days (mean: 7.6±0.15). As expected, lipid and gross energy were positively correlated with days-postpartum negatively correlated with time on shore (P<0.0005; all relationships). The preceding trip duration, however, had no effect on lipid (P=0.67), energy content (P=0.56), protein (P=0.33), or water (P=0.64). Likewise, dive type, maternal mass, and year showed no influence on the proximate composition of milk. Milk composition and previously published correlations between mean milk lipid (%) and mean trip durations are reviewed for otariids. Recently published data, including this study, do not support the hypothesis that increased time at sea increases milk lipid content in the Arctocephalinae otariids.

Introduction

Lactation is a fundamental defining character of mammalian reproduction. It is energetically the most costly period of the mammalian life cycle (Pond 1977, Clutton-Brock et al. 1989, Gittleman and Thompson 1988) and as such, it imposes strong selective pressures on behavior, ecology and life histories. Numerous behavioral and physiological adaptations have evolved (reviewed in Pond 1977) to facilitate the increased energetic demands of mothers and for rapid neonatal growth. Some of the most extreme adaptations regarding lactation are found in pinnipeds. The selective forces driving these adaptations are the result of time and energy constraints imposed by the temporal and spatial separation of terrestrial parturition and marine foraging (Costa 1993, Boyd 1998). As a result, pinnipeds have evolved energy-dense milks and some of the shortest lactation intervals on record for large mammals. Pinnipeds as a whole, however, show considerable diversity in lactation strategies and are thus useful for testing life history theories. Current energetic models used in testing of life history theories rely on information on milk composition and energy transfer between parent and offspring. Within the pinniped group there is both considerable inter- and intra-specific variability in the proximate composition and energy density. In otariid species, for example, there can be up to a three-fold range in the amount of lipid, the primary source of energy (18-56%, Antarctic fur seals, Arnould and Boyd 1995a; 14-58%, Australian fur seals, Arnould and Hindell 1999; 25-56%, Subantarctic fur seals, Georges et al. 2001; 7-43%, Australian sea lion, Kretzmann et al. 1991, Gales et al. 1996; 12-35%, Steller's sea lion, Adams 2000). Understanding the sources of intra-specific variability in proximate composition of milk is necessary for evaluating trends in life history patterns within the context of the mammalian phylogeny.

Table 1. The gross chemical composition of 189 milk samples from 101 northern fur seals sampled throughout lactation (July-October) on St. George and St. Paul Islands, AK in 1995 and 1996.

Milk Component ^a	Mean	SE	Range
Lipid (%)	49.07	0.56	28.50 - 66.85
Protein (%)	10.82	0.11	6.41 - 15.64
Ash (%)	0.49	0.01	0.16 - 1.20
Water (%)	37.61	0.55	20.17 - 62.43
Gross Energy ^b (kJ·g ⁻¹)	21.26	0.21	13.05 - 27.98

^aCompositions were not measured. ^bCalculated gross energy.
^cAs they are generally less than 1% of total mass, gross chemical composition of dried milk (20% dry) is shown.
^dCalculated using energy density values for lipid (39.6 kJ·g⁻¹) and protein (16.3 kJ·g⁻¹).

MATERIALS & METHODS

Study Area and Sample Collection

The study was conducted on the Pribilof Islands of St. Paul and St. George from July through November in 1995 and 1996. Milk was collected at 189 captures of 101 females. Samples of milk (20-15 mL) were manually expressed approximately 5-15 minutes after administering oxytocin (0.25 mL of 10 U/mL). In most cases, milk was easily collected from one teat; however, if milk flow was difficult or slow other teats were used until a full sample was obtained. Milk was collected in 15 mL screw cap plastic vials and was stored frozen (-20°C) within several hours of collection. Attendance data (time on shore and time at sea) were collected using VHF radio transmitters and an automated recording system.

Dive Data

Dive data was collected using time-depth recorders (TDRs; Wildlife Computers, Redwood, WA). TDRs (MK2, 140 x 20 mm, 150g; or a MK5, 64 x 38 x 13 mm, 50g) were attached to the peltage with five-minute epoxy mixed 60:40 ratio to hardware (Devcon). TDRs were programmed to record depth every 10 seconds and had a resolution of 1 meter. Data were analyzed for dives > two meters using Wildlife Computers dive analysis software (Zero-offset Correction, v1.22 and Dive Analysis, v4.08). Each dive record (i.e. each individual trip to sea) was then classified into one of three dive types (shallow, mixed, and deep) using the following criteria:

- Shallow: 90% or more of all dives <20 meters
- Deep: 60% or more of all dives >50 meters
- Mixed: All remaining records.

Sample Analysis

Before all analyses, samples were thawed in a warm water bath (40°C) and mixed thoroughly. All samples were analyzed in duplicate. Water content was measured by drying sub-samples in pre-weighed crucibles in a forced air convection oven at 100°C (for 1 h) overnight. Samples were weighed every 10 hours until a stable mass was achieved. Ash content was measured by incineration of the dried samples in a muffle furnace at 500°C for 24 hours (AACC, 1995). Total lipid was measured using the Roese-Gottlieb method (AACC, 1995). Due to the high lipid content of pinniped milk, we performed an sequential extraction to insure complete extraction of all lipid. Duplicate samples and within 3% of each other were reported. Total energy (TE) was measured with the Kjeldahl method and crude protein was calculated as TE x 0.16 (AACC, 1995). Non-protein nitrogen (NPN) was not measured as the few studies that have measured NPN of fur seals found that it typically comprised only 3.7% of TN or less than 0.1% of total sample mass (Adams et al. 1996, Othello 1984, Telford and Lachner 1986). Carbohydrates were not measured as they generally represent less than 1% of total mass in pinniped milk (Othello 1987). Previous studies of southern fur seal milk found carbohydrates to be 0.11% (Adams et al. 1996) and 0.4% (Othello et al. 1984). Gross energy was calculated using energy density values for lipid (39.6 kJ·g⁻¹) and protein (16.3 kJ·g⁻¹; Kitchner 1975).

Statistical Analysis

Analyses used a multiple linear regression model using continuous (days postpartum [DPP], time ashore [TAS], preceding trip duration [PTD] and maternal mass) and categorical variables (Year, Island, and Dive Type) (SYSTAT 10 2000). Unless otherwise indicated data are presented as mean ± SE and results were considered to be significant at P<0.05.

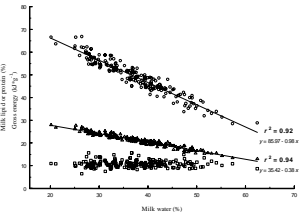


Figure 1. The relationships between water (%), lipid (%), protein (%), and gross energy (kJ·g⁻¹) in northern fur seal milk.

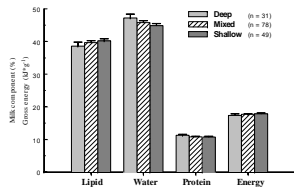


Figure 4. The composition of milk from northern fur seal females of different diving types and the gross energy of their milk. Lipid, water, and gross energy values were first adjusted for time spent on shore and days postpartum.

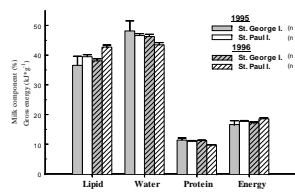


Figure 5. The composition of milk from northern fur seal females foraging from St. George and St. Paul Islands in 1995 and 1996. Lipid, water, and gross energy values were first adjusted for time spent on shore and days postpartum.

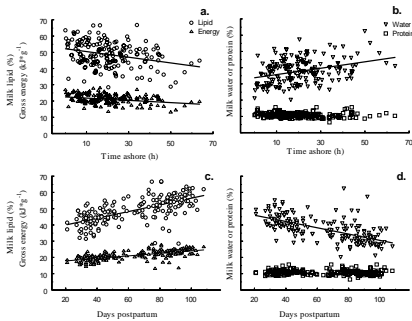


Figure 2. The relationships between: a, time ashore and milk lipid (%), and gross energy (kJ·g⁻¹); b, time ashore and protein (%), and water (%); c, days postpartum and lipid (%), and gross energy (kJ·g⁻¹); d, days postpartum and protein (%), and water (%).

Table 2. The level of significance (P-values), correlation coefficients and residual mean squares for five regression models examining the role of seven factors in influencing the proximate composition of northern fur seal milk.

Milk Component	Time on Shore (h)	Days Postpartum	Preceding Trip Duration (d)	Maternal Mass (kg)	Dive Type	Year*Isle	Residual Mean Square
Lipid (%)	<0.0005	<0.0005	0.65	0.14	0.11	0.23	0.64
Protein (%)	<0.0005	<0.0005	0.56	0.13	0.19	0.23	0.69
Water (%)	0.18	0.96	0.23	0.58	0.50	0.12	0.11
Ash (%)	0.04	<0.0005	0.19	0.61	0.33	0.98	0.38
Gross Energy ^a (kJ·g ⁻¹)	<0.0005	<0.0005	0.52	0.12	0.17	0.35	0.09

^aCalculated gross energy.

Results

Proximate Composition

A summary of the proximate composition of 189 milk samples is presented in Table 1. Lipid accounted for, on average, 49.1% (±0.56, n=189) of the gross chemical composition and 87.7% (±0.18, n=189) of the total calculated gross energy. Lipid values ranged from 28.5 to 66.8%. Protein accounted for 10.8% (±0.11, n=189) of the gross chemical composition and 14.1% (±0.11, n=189) of the total calculated gross energy. Ash comprised 0.5% (±0.01, n=189). On average, the sum of measured components (lipid, water, protein, and ash) accounted for 98.0% (±0.13, n=189). The total calculated gross energy was 21.3 kJ·g⁻¹ (±0.21, n=189). The percent lipid and gross energy were negatively correlated with water content (Lipid: P<0.0005, r²=0.92; Gross energy: P<0.0005, r²=0.94) whereas, protein did not correlate (r²=0.81, r²=0.001) with water (Figure 1). Regression equations were:

$$\text{lipid [\%]} = 85.97 - 0.98 \text{ water [\%]}$$
$$\text{gross energy [kJ·g}^{-1}\text{]} = 35.42 - 0.38 \text{ water [\%]}.$$

Sources of variability

Time ashore ranged from 0.03-63.63 hours (mean: 20.9 ± 13.1 hours; Figure 2a). Milk lipid and gross energy both had significant negative correlations (P<0.0005, Table 2) with time ashore (Figure 2a). On average females' milk had 0.160% less lipid and 0.1% less energy for every hour spent on shore. Water content was positively correlated (P<0.0005, Table 2; Figure 2b) but protein content was not affected by time ashore (P=0.18, Table 2; Figure 2b).

Days postpartum in our milk collections ranged from 0 to 108 days. Our sampling protocol resulted in a bi-modal distribution with the first mode at 35 days postpartum (mid-lactation) and the second mode during late-lactation at 91 days. Milk lipid and gross energy were positively correlated with days postpartum (P<0.0005, Table 2; Figure 2c) and water content had a negative correlation (P<0.0005, Table 2; Figure 2d). Days postpartum had no effect on milk protein (P=0.96, Table 2; Figure 2d).

The trip durations in our study ranged from 2.5 to 14.1 days and were normally distributed (Kolmogorov-Smirnov goodness of fit test: P=0.005). Mean trip duration was 7.57 (± 2.03) days. A female's preceding trip duration had no effect on any of the major milk components (Figures 3a & b; Table 2, lipid: P=0.65, water: P=0.56, protein: P=0.23) or on gross energy content (Figure 3a; Table 2, P=0.52).

Maternal mass averaged 39.4 (± 5.1) kg and was also normally distributed (Kolmogorov-Smirnov goodness of fit test: P=0.005). Maternal mass in this study had no effect on any of the major milk components (Figures 3c & d; Table 2, lipid: P=0.14, water: P=0.13, protein: P=0.58) or on gross energy content (Figure 3; Table 2, P=0.12).

Dive type had no influence on the proximate composition of milk or gross energy (Figure 4). There were differences in the mean percent lipid (deep divers: 46.8 ± 8.4, shallow divers: 50.9 ± 6.4, mixed divers: 48.8 ± 7.6), water (deep divers: 40.1 ± 8.1, shallow divers: 35.4 ± 6.4, mixed divers: 37.9 ± 7.3), and mean gross energy (deep divers: 20.5 ± 3.2 kJ·g⁻¹, shallow divers: 22.0 ± 2.4 kJ·g⁻¹, mixed divers: 21.1 ± 2.9 kJ·g⁻¹). However, the differences can be attributed to shallow diving being more frequent in late-lactation. Once values were adjusted for the significant variables, days postpartum and time ashore, the resulting adjusted values showed no significant differences (Table 2; lipid: P=0.11; water: P=0.19; protein: P=0.50; gross energy: P=0.17).

There were no differences in milk composition between 1995 and 1996 (Table 2; lipid, P=0.23; water, P=0.21; protein, P=0.12; gross energy, P=0.35; Figure 5).

Differences in milk composition did exist between St. Paul and St. George Islands. Milk lipid content in seals foraging from St. Paul Island in 1996 was greater (Table 2, P=0.05) than on St. George Island (Figure 5). There were no differences in water (P=0.25) or protein (P=0.11) content or in gross energy (P=0.09). There were no interactive effects between year and island (Table 2; lipid, P=0.64; water, P=0.69; protein, P=0.38; gross energy, P=0.75). After testing our multi-linear models, which considered all measured potential sources of variability, we repeated our analysis using only the significant terms. Coefficients for the significant terms are reported in Table 3 (not presented). Milk lipid content decreased with time ashore and increased with pup age (r²=0.55, lipid [%]=39.61-0.160[TAS]+0.191[DPP]), where TAS=time ashore and DPP=days postpartum). Water content increased with time ashore and decreased with pup age (r²=0.53, water [%]=45.87-0.187[TAS]-0.181[DPP]). Gross energy (kJ·g⁻¹) of milk decreased with time on shore and increased with pup age (r²=0.50, Energy [kJ·g⁻¹]=17.80-0.064[TAS]+0.075[DPP]).

Conclusions

- Studies of proximate composition of milk must consider stage of lactation (days postpartum) and length of suckling bouts (time ashore for Otariids)
- Preceding trip duration does not influence the proximate composition of milk in Otariids
- Dive pattern (i.e. diet) also does not influence proximate composition of milk (milk yield was not measured in this study)

Acknowledgments

We are most grateful to Drs. T. Loughlin and G. Antonellis, J. Sterling, J. Baker, R. Ream, B. Robson of the National Marine Mammal Lab, Seattle, WA. This study benefited from discussions with milk composition with Dr. Sara Iverson, Dalhousie University. Funding for this study was provided by a National Science Foundation grant, (OPP-950072) to D. Costa and M. Goebel and support through from the National Marine Fisheries Service.



Figure 3. The relationships between: a, the preceding trip duration and milk lipid (%), and gross energy (kJ·g⁻¹); b, trip duration and protein (%), and water (%); c, maternal mass and lipid (%), and gross energy (kJ·g⁻¹); d, maternal mass and protein (%), and water (%).